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Impact of chemical modifications in pilot-scale isolated sorghum starch and commercial cassava starch

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Abstract

The aim of this work was to evaluate the impact of different chemical modification methods on starch from different botanical sources. Brown and white sorghum starch were isolated throughout a time saving process and avoiding the use of dangerous compounds. Brown and white sorghum and commercial cassava starch were treated using acetic acid, acetic anhydride and octanoyl chloride. Its chemical, morphological and thermal characterization were afterwards carried out. The modifications reduced amylose content and increased damaged starch. Both acetylation and acid treatment produced no significant changes in the size and shape of granules; yet, they increased superficial pores. SEM observations supported the results, indicating that octanoyl modification causes a partial destruction of the granule structure. Thus, particle size distribution changes significantly. Crystallinity degree decreased with all the modifications. However, the effect was more pronounced in octanoyl esterification. In general, modifications increase the water absorption of the native starch but no considerable effect was found over oil absorption, and gelatinization parameters were significantly altered as a result of modifications. Starch from these subtilized sources was successfully modified, which could arouse interest in its industrial production.

Keywords

Sorghum starch, cassava starch; pilot-scale; chemical modification

1. Introduction

The most common sources of food starch are corn, potato, wheat, cassava and rice. Sorghum and cassava are cultivated in economically depressed regions and become a fundamental resource for these countries. Its economy could be strengthened by converting this relatively low-cost raw material into high value starch.

Sorghum (*Sorghum bicolor (L.)* Moench) is a cereal crop; however, unlike the four cereals most widely produced worldwide (maize, wheat, rice and barley), it is capable of growing in drought conditions, salty soils and other adverse conditions with acceptable yield levels. Considering that,

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and its low-water and fertilizer requirements and high efficiency in solar energy conversion, sorghum becomes a sustainable crop [1,2]. In addition to its agronomic properties, sorghum has around 70 % (d.b.) of starch and its external layers contain a wide variety of polyphenols with well-known antioxidant activity. These layers determine grain color, where brown hybrids have higher amounts of polyphenols (mostly as tannins) than the white ones, preventing starch isolation [3,4]. These properties make sorghum a particularly promising alternative to human consumption worldwide. Despite that, production is mostly used for animal feeding and scarcely in the food industry.

Many improvements have been made in the separation of sorghum starch on a laboratory scale and numerous methodologies have been proposed to achieve this effectively [3,5,6]. In general, all these isolation methods adopt a time-consuming steeping stage and are applied only at a laboratory scale.

In a previous work [7], we investigated several sorghum hybrids grown in Argentina where we could demonstrate that grain color did not influence the chemical composition of flour, nor its thermal and physicochemical properties. These allow the selection of hybrids with different characteristics, highlighting the versatility of sorghum flour as a constituent for food and non-food applications.

On the other hand, cassava (*Manihot esculenta* Crantz) is a perennial shrub whose fresh roots contain high amounts of starch (about 30 %); it is one of the most important food crops in the tropics [8,9]. The short storage time of cassava requires fast consumption or processing as flour or starch [10]. Cassava starch can be easily isolated, having numerous food applications. Cassava starch is a key ingredient for food industry and also for other kinds of applications due to its particular physicochemical behavior (high clarity and high viscosity pastes, low gelatinization temperature and low tendency to retrogradation) [11]. Yet, its functional properties are limited; that is why several authors have studied cassava starch modifications [10,12].

The chemical modifications of native starch were investigated for many years led by the discovery of new applications, improvements in the processes and novelty of substitution groups. In order to

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avoid expensive methods and the use of toxic solvents, modifications with low degree of substitution were adopted over the past years [13].

Starch esterification is one of the most common chemical modifications, which implies substitution of a hydroxyl group. In this sense, different acetylation methods (substitution with acetyl groups) were performed on a wide range of starches in aqueous media, the industrially-produced maize starch being the most common raw material [14]. Singh et al. [15] isolated sorghum starch by a wet milling method that includes NaOH steeping stage; the starch obtained was modified using acetic anhydride at various levels.

Fang et al. [13] also developed a simple and economical method for the preparation of esterified maize starch where reaction with octanoyl chloride yielded the most successful results after screening a wide range of reaction conditions. Octanoyl chloride is not approved by FDA, this modified starch could be used in other applications such as paper and bioplastic development [22]. Despite this, the method was selected because represents a simple, economic and environmentally friendly alternative procedure for the preparation esterified starch [13].

Starch treatment with acid produces chain hydrolysis, most frequently in the surface and amorphous regions. Its crystallinity is then modified but its granule shape is not [16]. Acid-modified starch is usually prepared with mineral acids during different times, usually for days [17]. These time-consuming treatments, in addition to the risks involved in handling strong inorganic acids and their undesirable by-products, should be taken into account. For this reason, Babu et al. [18] treated sweet potatoes with organic and inorganic acids under gentle conditions, reaching good esterification results.

The aims of the present work were to isolate sorghum starch from two different hybrids at pilot scale level and to modify sorghum starches using environmentally friendly approaches. In this research a novel sorghum starch isolation method was employed on two different hybrids followed by chemical modification of starches. The chemical-, morphological-, absorptive- and thermal-properties of starch samples were evaluated. Cassava starch is an economically important starch for Asia, Africa and South America and it was also modified following the same procedures and

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the obtained results compared with those from sorghum. These results may be useful in finding new applications for sorghum starch in food and non-food productions.

2. Materials and methods

2.1. Materials

White and brown sorghum flour (hybrids Pannar-8706 W and Pioneer-81G67, respectively) was produced at an industrial scale in Amylum S.A. facilities (Córdoba, Argentina) through decortication of grains with a vertical dehulling device (MNMLs 3, Green Horse, China) at a weight loss of 10 % and then milled in an industrial hammer mill (Bartoli Hnos., Argentina) with 125 HP motor and 0.4 mm triangular mesh coupled to a sieve with 0.2 mm single channel mesh. White sorghum flour composition (db) was: ash 1.2 %, lipid 5.4 %, protein 7.9 %, and carbohydrates 85.5 %; and brown sorghum flour was: ash 1.3 %, lipid 5.4 %, protein 8.7 %, and carbohydrates 84.6 % [19].

Cassava starch was bought from local market (Montecarlo Coop., Argentina).

Acetic acid, acetic anhydride (Anedra S.A., Argentina) and octanoyl chloride (Sigma-Aldrich, USA) were analytical reagents. All the other chemicals were analytical grade reagent and all solutions were prepared with deionized water.

2.2. Sorghum starch isolation

Starch isolation was performed by wet-milling, separating the other components from the grain, namely, germ, bran, fiber and protein. It should be noted that decorticated flour contains low levels of bran and germ. Sorghum proteins are mainly prolamins packed as spherical bodies and tightly bound to starch granules [20], so proteins was detached using high energy wet milling and separated mainly in the sedimentation table.

For the sorghum starch isolation, 1 kg of flour was placed in 3 L of water in a colloidal mill (MCE 35, Colmill, Brazil) and finely ground for 15 min. The suspension was filtered, to separate bran and germ, through an 80 wire sieve (177 µm), adapted to a drum centrifuge (Padrão C-I, GV Maquinas, Brazil). The mesh was washed with 3 L of water, discarding the material retained. This

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procedure was repeated with a 270 sieve (53 µm) and the resulting suspension was allowed to settle for 1 h, after which the supernatant was discarded and the sediment was resuspended in 3 L of water. This slurry was pumped into a sedimentation table and the solids retained were collected to be dried at 50 ºC overnight.

2.3. Proximal composition of native starch

Moisture, ash and protein quantification was carried out by standard methods [21]. Total starch was determined following the AACC Method 76-13.01 using the K-TSTA assay kit (Megazyme International, Ireland).

2.4. Chemical modification

2.4.1. Acid treatment

The acid treatment to accomplish acetylated starch was based on the procedure described by Babu et al. [18] with modifications. Starch was dispersed in 5% acetic acid for 1 h at 45 °C in a water bath with constant magnetic stirring. The pH was then adjusted to 7.0 with 0.1 M NaOH. The acid-treated starch (A) was collected by filtration and washed three times with deionized water. It was finally dried at 50 °C overnight.

2.4.2. Acetylation by acetic anhydride

The starch acetylation was performed using the method described by Singh et al.[15] with modifications. Starch was dispersed in water (35% w/w) and 0.75 M NaOH was added until pH 8. Afterwards, acetic anhydride was added (1 g /100 g of starch) dropwise under constant stirring at pH 8 with NaOH. Once the anhydride was completely added, the reaction was allowed to proceed for 10 min. Then pH was adjusted to 4.5 with 0.5N HCl and the starch was collected by filtration with posterior washes with water and 96% ethanol. Finally, acetylated starch with acetic anhydride (H) was dried at 50 ºC overnight.

2.4.3. Octanoyl esterification

The acylation of gelatinized starch chains with octanoyl chloride was carried out following the process proposed by Fang et al.[13] with modifications. Starch was suspended in water (5 % w/w) and 2.5 M NaOH was added under a nitrogen atmosphere until pH 10 under mechanical stirring.

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After 30 min, the octanoyl chloride was added dropwise (0.5 mol/ glucosyl mol) and the reaction was performed for 1 h. Once the reaction was completed, the suspension was neutralized to pH 7 with acetic acid. The product was precipitated by addition of 96% ethanol and later collected by filtration and washed twice with ethanol. After filtration, residual ethanol was removed by evaporation in air and the octanoyl esterified starch (O) was dried at 50 ºC overnight.

2.5. Degree of substitution

The degree of substitution (DS) is defined as the average number of substituent group per glucose unit. The DS of starch was determined by titration following the method of Singh et al.[15]. Modified starch (0.5 g, dry weight) was accurately weighed and dispersed by stirring for 30 min at 50 ºC in 25 ml of 75% ethanol solution. The solution was then cooled, 10 ml of 0.5 M KOH was added, and excess alkali was titrated with 0.5 M HCl using phenolphthalein as an indicator. The blank was native starch used as a sample and the DS was calculated by Equation 1.

$$DS = \frac{162\ C\ (B-S)}{W-M\ C\ (B-S)} \quad (1)$$

where *C* is the molarity of HCl solution, *B* and *S* are the titration volume of HCl solution (ml) consumed in native starch and acid starch modified, respectively. *W* is the dry weight (mg) of the starch modified and M is the molecular weight of each substituent group (43 and 144 g mol^{-1} for acetyl and octanoyl group, respectively).

2.6. Chemical properties of modified starch

The amylose/amylopectin proportion was analyzed with a commercial assay kit (K-AMYL, Megazyme International, Ireland), based on the method of Gibson et al. [23]. Resistant starch was measured according to AACC 32-40 [24] with an assay kit (K-RSTARCH, Megazyme International, Ireland) and expressed as g of resistant starch per 100 g of starch. Damaged starch levels were estimated following the American Association of Cereal Chemists method 76-30A. Later, the reducing sugars were measured (method 80-60; AACC). Damaged starch is defined as the grams of starch subject to enzymatic hydrolysis per 100 g of sample.

2.7. Fourier transform infrared (FT-IR)

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The FT-IR spectra were obtained by a Nicolet iN10 infrared microscope with a cooled detector (Thermo Scientific, USA) operating between 650 and 4000 cm^{-1} and a resolution of 8 cm^{-1}. Freeze-dried samples were placed over KBr discs and the spectra were collected and analyzed with OMNIC™ software (Thermo Scientific, USA).

2.8. Starch powder color

The color of powder starch was measured with a spectrophotometer (CM-600d; Konica Minolta, Japan) using a D65 illuminant at 10˚observation. The results were expressed in the CIELAB scale (*L*, a*, b**) and compared through whiteness index.

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (2)$$

2.9. Particle size distribution

The particle size distribution of starch samples was measured in a laser particle size analyzer (LA-960, Horiba Instruments, USA) using the built-in liquid sampler equipped with a sonicator. The samples were suspended in water with both circulation speed and stirring rate set at level 10 (of 15). The refractive indices used were 1.54 for the starch samples and 1.33 for the dispersant.

2.10. X-ray diffraction (XRD)

The crystal structure of starch was analyzed with an X-ray diffractometer (PW-1800, Philips, USA) with measurement conditions of 40KV and 30mA using time steps of 2.5 s and angular step of 0.02º to scan from 2 to 30º. The crystallinity degree (CD, ratio of crystalline/amorphous phase expressed as percentage) was calculated through diffractogram deconvolution (Ribotta, Cuffini, León & Añon, 2004) using Peakfit software v4 (Jandel Scientific, USA).

2.11. Scanning electron microscopy (SEM)

The morphology of starch granules was analyzed through images taken with a scanning electron microscope (Sigma, Carl Zeiss, Germany) at an electron acceleration of 3 kV under high-vacuum conditions and magnification of 4000 x were used for images acquisition. For that, starch samples were placed over aluminum stubs with carbon sticky tape and coated with a thin layer of gold. In order to quantify the microstructure differences between starch samples, porous size and porous

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surface proportion were evaluated. Images were analyzed using the open source software ImageJ (v1.51s, National Institutes of Health, USA).

2.12. Water and oil absorption

The water and oil absorptive capacity of starch was determined following the procedure reported by Yousif et al.[26]. Starch sample was suspended into the solvent (water or sunflower oil) at room temperature. It was then centrifuged and the supernatant discarded. The sediment was weighed and the absorption was calculated as g of absorbed solvent by g of starch sample.

2.13. Thermal properties

Thermograms were obtained with a differential scanning calorimeter (DSC 823e, Mettler Toledo, Switzerland) and evaluated by STARe software. Starch (10 mg db) and 20 µL of deionized water were introduced into 100 µL aluminum pans and hermetically sealed. After 24 h at room temperature, pans were heated from 20 to 120 ºC at a heating rate of 10 ºC/min. Thermogram parameters assessed were: onset temperature (To), peak width and gelatinization enthalpy (ΔH, J/g of starch).

2.14. Statistical analysis

All assays were carried out at least in duplicate. The analysis of variance (ANOVA, multiple comparison test by DGC method, α=5 %) and Pearson's correlation coefficients (*r*) were performed using InfoStat software (Version 13p, [27]) and the graphics were made using Excel software (2016 Version, Microsoft).

3. Results and discussions

3.1. Proximal composition of native starch

Sorghum-isolated white starch exhibited 95.1±1.2% of starch (db) and brown sorghum isolate presented 96.7±1.0% of total starch (db). For both sorghum starch isolates non starch polysaccharides, protein, ash and lipid content were lower than 2.9, 0.5, 0.2 and 1.5 %, respectively, and starch recovery ranged from 65 to 72 %.

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Considering these results, starch purity values could be regarded high as compared to previous articles [3–5]. These authors performed long alkali or sulfur dioxide steeping and reported isolation recoveries between 27.7 and 80.4 %, with purity from 93 to 99 %.

The isolation method developed was based only on physical separations and did not require any reagent nor long-time grain steeping. On the other hand, cassava commercial starch shown a total starch content of 92.3% (db).

3.2. Degree of substitution of esterified starch

The degree of substitution (DS) indicates the amount of hydroxyl replaced for other groups per glucose unit in the starch polymers. All esterified samples presented low DS, in agreement with the objective of the present work, with values around 0.016 for sorghum starch and between 0.016 and 0.050 for cassava samples (Table 1). The A samples revealed no significant differences in relation to DS values of native starch, showing that no esterification was produced by acetic acid treatment.

The DS values of H samples were lower than expected, as compared with similar reaction conditions reported by other researchers [15]. These DS values could result from a low porosity of the granule surface, hindering the reactive access to the granule interior and influencing the extent of the reaction [28].

The O samples also presented lower DS values than those reported in their original work [13]. This could be due to the lack of catalyst, the different method to determine DS and the different botanical sources for the modification. Sorghum starch also presented lower DS when compared with cassava sample, indicating greater susceptibility of root granules than that found in cereal ones.

3.3. Amylose content

All the modification performed in this research decreased the amylose content in relation to that of native starch (Table 1), where treatment with acetic anhydride caused the largest decrease. Starch amorphous phase and amylose were more susceptible to react compared to amylopectin

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and crystalline region [29,30]. Then the amylose reduction during the modification process was probably due to the acid or alkali hydrolysis occurred mainly on the amylose chains.

On the other hand, the amylose content in cassava starch was slightly lower than that in sorghum starch for each modification. This could be ascribed to the weaker structure of the former and to differences in the amylose chain extent [31].

3.4. Resistant starch

Resistant starch is the portion that is not hydrolyzed after 120 min and can be classified into four types; one of them is the type 4 which has become partially indigestible due to strong oxidation, etherification or esterification treatments [32].

The resistant starch (RS) content of all starch samples was low (< 5 %) and showed slight changes as a result of the chemical modifications (Table 1). As expected, these chemical modifications did not increase the RS content because of the partial hydrolysis produced during the modification process.

In addition, in almost all cases, the cassava samples showed higher RS content than that found in sorghum ones, probably because native tuber, roots and legumes starch have amylopectin with a higher proportion of long chain branches, resulting in a more compact structure (RS type 2) [32].

3.5. Damaged starch content

The evaluation of damaged starch is central for characterizing raw material since it determines the properties of the final product. Thus, it is used as an indicator of the milling process in cereal flour or of the starch isolation method [33,34].

The native samples of sorghum starch showed low amount of damaged starch (Table 1), indicating that the starch isolation method proposed in this research could produce starch with low granule damage.

Mbougueng et al. [10] reported a wide range of damage levels in the granules produced by starch acetylation, but the evaluation was carried out only through SEM images. In the present work both SEM (see below for further discussion) and chemical evaluation of damaged starch showed that the acetylation procedure slightly modified the amount of damaged starch (Table 1). These

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behaviors were also observed for acid-treated samples indicating low damage of the granule surface.

On the other hand, starch damage was increased during obtention of O due to the alkali hydrolysis of starch. These results were consistent with the changes found in SEM images and XRD pattern analysis (see below for further discussion).

3.6. Fourier transform infrared (FT-IR)

Investigation and interpretation of FTIR spectra of starch allow chemical characterization through the identification of new bonds created by reactions and the presence of unreacted chemicals. For this, the spectra of the modified starch were compared with those of native starch and also with previous publications of other researchers [13,18,35–38]. Although the analysis was performed for all the samples, Fig. 1 only shows spectra of native and modified brown sorghum starch since each reaction produced the same effects over the other types of native starch.

In the esterified starch samples (H and O), an ester band signal (1740 cm^{-1}) was exhibited, and the presence of this band was not detected in native starch; thus, the substitution reaction occurred in both cases. In O samples, the absence of peaks at 1800 cm^{-1} and 1700 cm^{-1} implied absence of chlorides or caprylic acid [13]. However, at 1640 cm^{-1} the presence of a peak could indicate that acid salts were detected, but as this peak was also found in the native starch spectrum, then it was probably due to water strongly bound to starch. In the fingerprint region (<1100 cm^{-1}) differences with the other samples were observed due to the partial gelatinization of starch that changed C-O bond stretching. These results were similar to those reported by Fang et al. [13].

The FTIR analysis suggests that both acetylation and acid treatment were free of unreacted reagents: acetic anhydride with the absence of peaks in the 1850-1760 cm^{-1} region and acetic acid through lack of absorption in 1700 cm^{-1}. These observations were in agreement with those observed by Colussi et al. [37].

3.7. Starch powder color

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Color is one of the most important sensory parameters for consumers and, consequently, for the food industry. The color parameter values for isolated sorghum starch presented high L* (92.48 and 91.73 for white and brown sorghum, respectively) and low chromatic parameters (a* 0.63 and 0.34 and b* 5.28 and 4.98 for white and brown sorghum, respectively). Other authors [3,5,39] reported problems with the separation of polyphenols, which colored their final products. The procedure proposed in this research allowed the successful separation of the compounds that give color to sorghum by polish the grains prior to grinding and starch separation process.

Color analysis of the powder samples showed that modifications produced no significant effect on the measured parameters a* and b*, with average values 0.33±0.33, and 4.82±0.76, respectively. The L* parameter exhibited slight but significant changes as a result of modifications, presenting an average value of 92.60. The acid treatment produced a decrease in L* value, probably due to an increase in surface roughness. Conversely, the L* parameter significantly increased between 1.6 and 0.5 after the anhydride acetylation of native starches, as opposed to what reported [10]. However, these differences were slightly noticeable or barely perceived by human eye according to the findings reported by Yamauchi [40] and verified by Khan et al. [1].

In this sense, WI values show significant differences among samples ranging between 88.8 and 92.6. However, these results did not show a clear trend in relation to the modifications.

3.8. Particle size distribution

Particles with different size usually have dissimilar hydrothermal response, water absorption and digestion kinetics, then, elucidation of particle size distribution is critical [41,42]. Granule size of native starch ranged between 5 and 34 μm, with a normal distribution. Both types of native sorghum starch had similar median, mean and mode but were slightly larger than cassava starch (Table 1).

Even though Fornal et al.[43] observed that a slight decrease in starch granule size could be produced during acetylation treatment, in our samples, acetylation produced no significant

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changes in granule size, as previously reported by Ali and Hasnain [2] for low degree acetylation starch.

In addition, no significant differences were observed in the granule size distribution between the native and A starch. These results were in agreement with those reported by Babu et al.[18], who used organic and inorganic acids at low concentrations. Conversely, Palma-Rodriguez et al. [16] reported substantial changes in size distribution of rice and maize acid-modified starch by high concentrations of sulfuric acid.

Octanoyl modification process partially destroyed granule structure, hence, particle size distribution suffered a severe change in relation to each native starch. The distributions changed the normal curve profile to a wide plateau shape spreading up to ≈400 μm. As a consequence, the span rises significantly (Table 1) indicating low homogeneity in starch particles. Cassava starch was the most affected one, probably because root and tuber starch shows weak internal granule organization [31].

3.9. Microscopic analysis

The SEM images (Fig. 2) were used to investigate the shape and surface characteristics of native granules and the effect caused by the different modifications. Sorghum native starch exhibited different types of granule shape: some were spherical having smooth surfaces and others were polyhedral with flat faces, according to the description reported by several authors [2,44]. A few granules could show a depression in the edges where protein bodies used to be (Fig. 2), as previously reported by Belhadi et al. [3].

Cassava starch granules were rounded and truncated, showing a smooth surface without pores, in agreement with previous reports of similar commercial products and laboratory-scale isolated starch [10,45]. The cassava acetylated and acid-thinned samples showed no differences in the granule surface, as compared with those in the native starch.

Olayinka et al. and Singh et al. [15,30] described that acetic anhydride starch modification produced a granule surface alteration due to gelatinization during addition of NaOH. However, the

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samples treated in the same way did not exhibit loss in surface smoothness, as described by these authors.

Although acetylation did not modify surface smoothness, sorghum starch pore size and quantity were increased. The same behavior was observed for both sorghums A samples, where the diameter of pores ranged between 37 and 809 nm with a pore area up to 2.4 % with respect to total granule surface. The H treated samples showed values slightly higher, presenting a minimum pore diameter of 68 nm and a maximum of 842 nm, reaching a relative area of 6.9 %. These differences probably arouse since the process of obtaining acetylated starches was more aggressive.

Pores and channels in starch were thoroughly studied by different authors, reporting its differential characteristics and the growing interest evidenced [2,30,46,47]. The porous granules have channels which connect the surface to its interior, generating a large specific area which allows penetration of reagents and, consequently, influence of the rate and extent of chemical and physical transformations.

The O samples exhibited partial destruction of starch structure (Fig 2) throughout the increase in surface roughness, fusion of starch granules and loss of granule identity. The alkaline treatment of starch produced similar transformations to that observed in physical methods like drum drying pregelatinization or extrusion cooking [48].

Observing the SEM images, the weaker structure of cassava compared with that of sorghum starch was evident. In this sense, different susceptibility to alkaline treatment for various types of starch was reported and depends of the granular structure complexity and the molecular architecture [49].

3.10. X-ray diffraction (XRD)

The X-ray diffraction (XRD) patterns have allowed characterization of the crystalline structure of granules and also quantification of amorphous/crystalline proportion, which is important to explain the thermal and rheological behavior of starch. The crystallinity of starch was related to

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amylopectin spatial arrangement to form clusters and, to a lesser extent, to amylose. Then any disruption causes reduction in the crystallinity degree [50].

All samples exhibited an A-type XRD pattern with main reflections (2θ) at 15°, 17°, 18° and 23° and weak peaks at 20° and 27° as reported for sorghum and cassava starch [8,51]. No presence of new peaks was detected in the modified starch, indicating that no new crystalline regions were formed. Also, each modification produces similar transformations on the native starch XRD patterns. As a consequence, only brown sorghum starch is shown in Fig. 3.

Fig. 3 shows a reduction of intensity in the XRD pattern of acetylated starch. Acetylation consists of addition of an acetyl group into starch chains, causing an interruption of the intra- and intermolecular hydrogen bonds that result in a weaker arrangement of amylopectin. Singh et al.)[15] did not find any significant change in the diffraction patterns of acetic anhydride modified starch. Yet, in agreement with the results reported by Lin et al. [45] for rosin acid-modified starches, changes in the XRD pattern were found for cassava and sorghum esterified starch.

On the other hand, acid-treated samples also exhibited slightly lower XRD peaks, probably due to depolymerization of the starch chains as reviewed by Hoover [17].

The peaks of modified starch were lower than those of native starch and, in consequence, crystallinity degree was reduced due to modifications (Table 2). In this sense, octanoyl substituent volume and granule degradation were reflected in its low CD. The modifications produced damage in starch granules, and therefore, CD exhibited a negative correlation with damaged starch ($p<0.05$, $r=-0.88$).

3.11. Water and oil absorption properties

The introduction of functional groups into the starch molecular structure alters its hydrophilic and lipophilic character. Then, modified starch absorption properties will depend on the nature of substituent, substitution degree, starch source, solvent and temperature [13]. Table 2 summarizes the water and oil absorptive capacity (WAC and OAC, respectively) of starch. In general, all modifications performed in the present work increase the water absorption of native starch. In particular, acetylation slightly increases water absorption in brown sorghum and cassava starch

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and has no effect in white sorghum starch. On the other hand, acetic acid treatment caused the major increase in water absorption in white sorghum starch, a slight increase in brown sorghum starch and no significant difference in cassava native starch. The behavior described could be explained by the increase in the surface area promoted by the superficial pores, as reported previously for these starches, which agrees with results found by Benavent-Gil and Rosell [46]. NaOH and acid hydrolysis might also increase the amount of low-molecular-weight starch chains, whose hydroxyl groups allow the formation of more hydrogen bonds and consequently enhance water holding capacity [18].

The OAC of acetylated starch was only significantly increased in white sorghum starch samples. It is probable that the greater amount of amylose content in these samples enhanced their ability to bond oil molecules throughout the formation of amylose-lipid complexes.

It is known that starch damage facilitates granules water swelling, i.e., compared to native starch, damaged starch could absorb up to 10-fold the amount of water [52]. Thus, water absorption showed a significant and high correlation (r=0.96, p<0.001) with damaged starch content. In this sense, the octanoyl-modified starch absorbs significantly more water and oil than the native starch, mainly due to starch damage produced during the modification method. This process also increases granule size, and consequently, particle surface, which produces a higher starch area exposed to water and oil contact.

3.12. Thermal properties

Gelatinization parameters of sorghum and cassava starch were significantly altered throughout esterification. Yet, no significant effect was seen in sorghum starch through acid treatment and cassava and brown sorghum acetylated starch. (Table 2). Gelatinization enthalpy indicates the amount of energy required for starch gelatinization and is related to the double helix arrangement and crystallinity changes [53,54]. In native starch, this parameter was within the normally reported range [4,44,51]. The ΔH of sorghum native starch were similar to those found in a previous work [7] in flour made from these hybrids.

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A decrease in gelatinization enthalpy produced by acetylation was usually reported for different starch sources [10,14,15], although a slight increase in ΔH was found in a few samples.

The ΔH of the octanoyl-esterified starch were lower than those of native starch, probably due to the destruction of the granule structure during NaOH treatment, reflected by a negative correlation with damaged starch (r=-0.68, p<0.01). This treatment produces a gelatinization degree of around 35 %.

The modifications did not alter the gelatinization temperature of white sorghum samples; however, slight changes were produced in sorghum and cassava samples. Despite this, the peak width was significantly affected during modifications. Cassava starch showed the highest peak width, which implied lower crystal perfection and heterogeneity of amorphous starch granules and crystalline regions [55].

Regarding acetic acid-treated sorghum starch, these samples showed the narrowest peaks. These results could be related to chain reorganization during the modification process, as reported for annealing methods where starch slurry was heated below its gelatinization temperature for a long time [56,57]; probably, the less organized chains were more susceptible to acid action.

4. Conclusions

The pilot scale isolation method of sorghum starch was effective for both sorghum hybrids considering the purity, damaged starch levels and color parameters. The modifications showed significant differences between cassava and sorghum starch and, in turn, both types of sorghum starch exhibited similar behavior. These treatments generate significant modification in starch considering chemical and morphological properties. In general, the modifications, and particularly, octanoyl esterification, causes significant changes in damaged starch, particle size distribution, granule shape, crystallinity degree and XDR pattern. In addition, modifications increased the water absorption of native starch; yet, no considerable effect was produced over oil absorption. Gelatinization parameters was significantly altered during modifications, particularly by octanoyl esterification. The chemical modifications applied under aqueous conditions are a powerful instrument to modify the chemical, morphological, absorptive and thermal properties. The

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relationship between these findings to end use and application of the modified starch is the main subject of a future research through the evaluation of their functional properties.

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6. Author Contributions

PM Palavecino carried out the assays, drafted the manuscript, and discuss the results; PD Ribotta and MC Penci made the experimental design and helps with results interpretation.

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8. Figures

Fig 1. FTIR spectra of native (NB) and modified brown sorghum starch: acetic anhydride (HB), acetic acid (AB) and octanoyl chloride (OB).

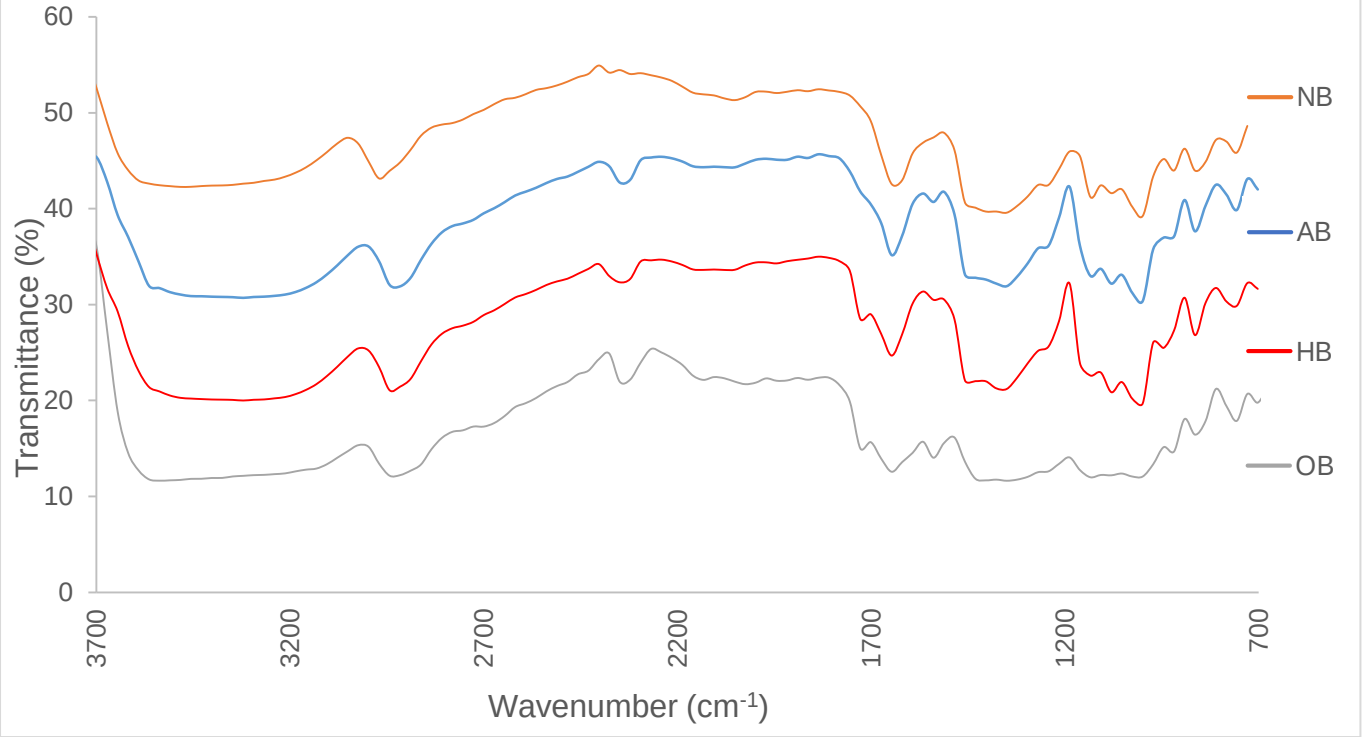

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Fig 2. Scanning electron microscopy (SEM) of native and modified starch.

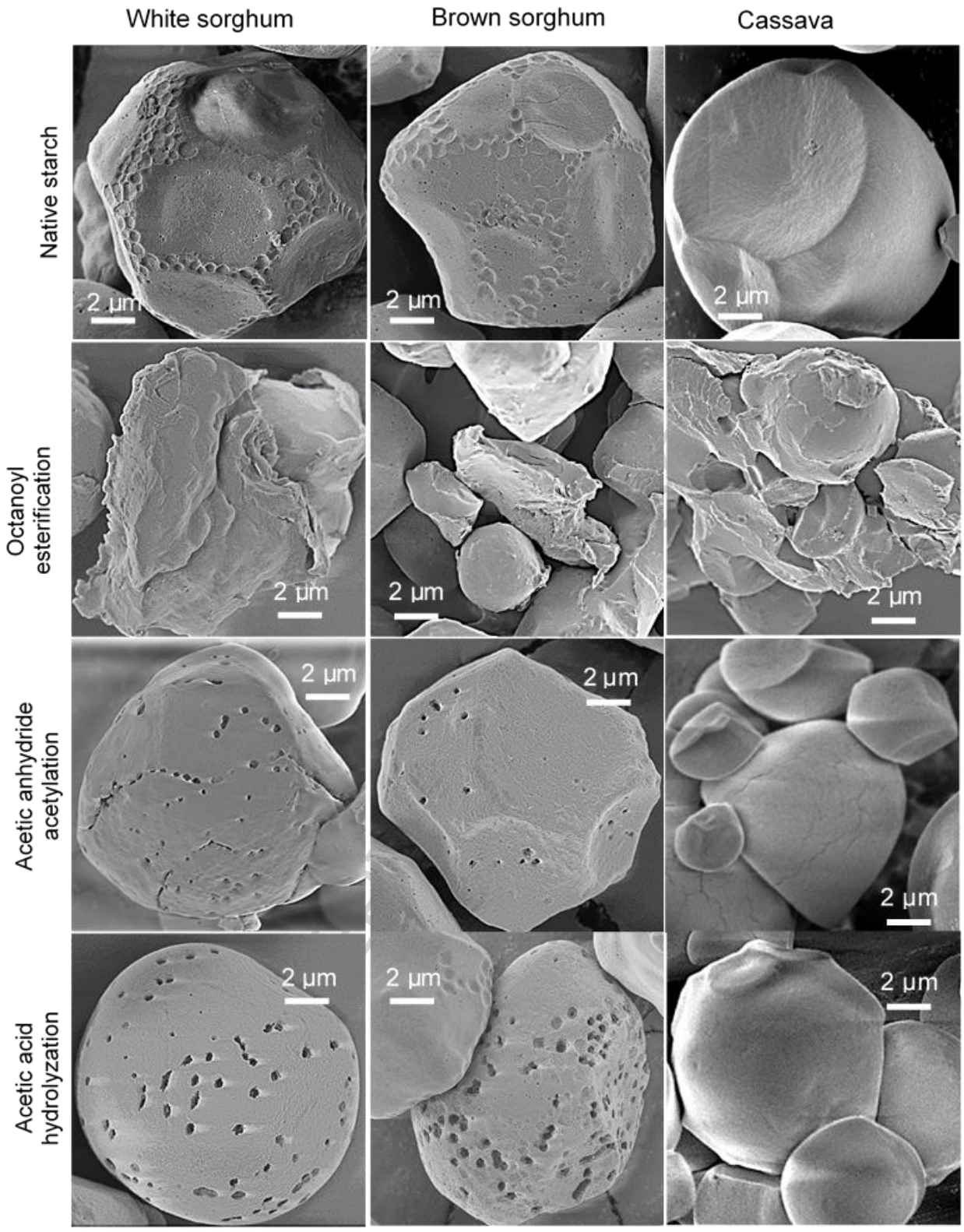

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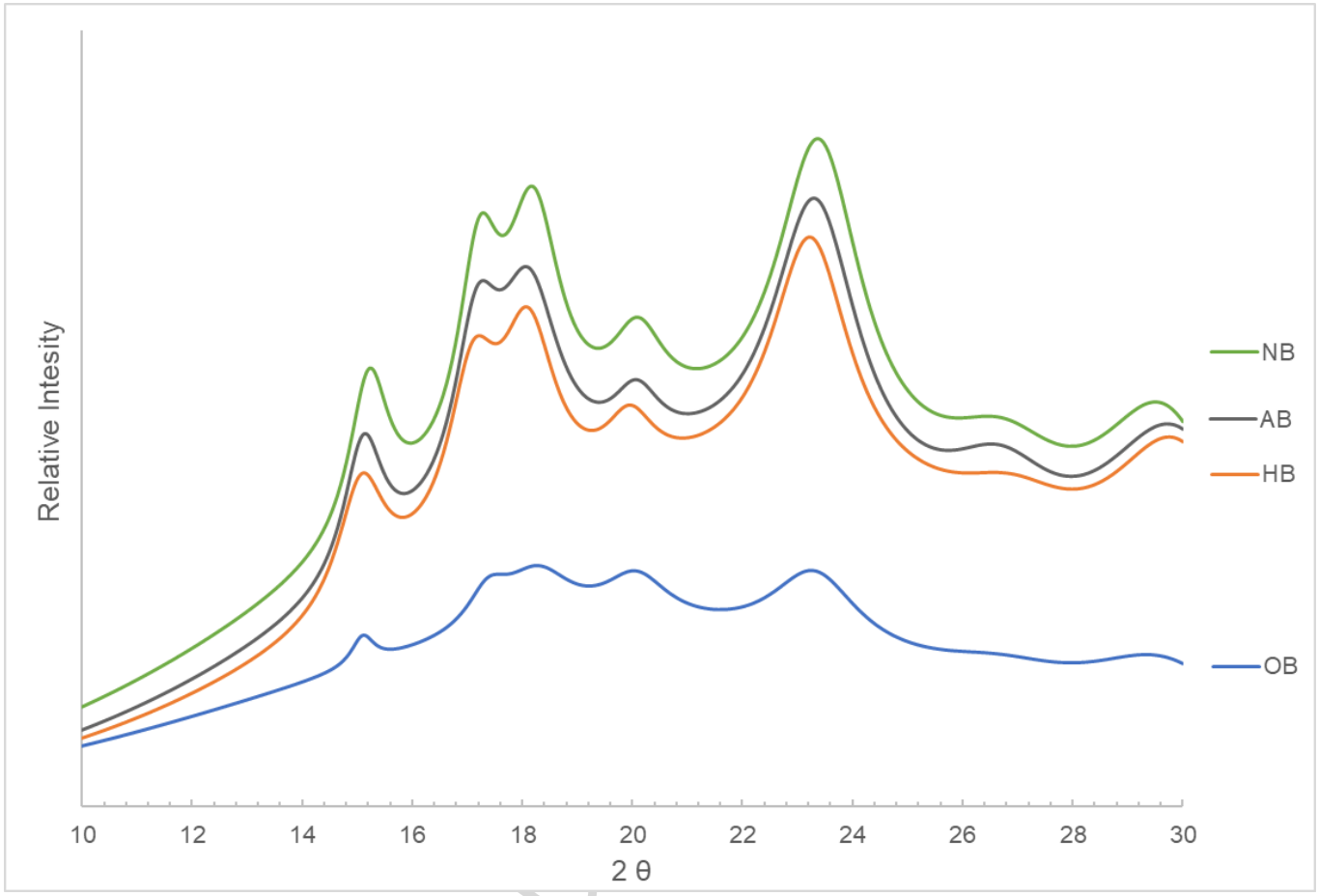
Fig 3. X-ray diffraction pattern of brown sorghum native (NB) and modified starch: acetic anhydride (HB), acetic acid (AB) and octanoyl chloride (OB).



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9. Tables

Table 1. Effect of chemical modifications on the chemical properties and the parameters of size distribution of sorghum and cassava starch.

Modifications	**Starch source**	**DS**	**AM** (%)	**RS** (%)	**SD** (%)	**Median size** (µm)	**Mean size** (µm)	**Mode size** (µm)	**Span**	**Dv10** (µm)	**Dv90** (µm)
Native starch	White sorghum		23.3^{d}	1.1^{b}	1.7^{a}	16.1^{b}	16.6^{b}	16.3^{b}	0.729^{a}	10.9^{b}	16.1^{b}
	Brown sorghum		21.3^{c}	1.5^{c}	1.7^{a}	16.2^{b}	16.6^{b}	16.3^{b}	0.691^{a}	11.2^{b}	16.2^{b}
	Cassava		24.0^{d}	4.7^{e}	2.7^{b}	13.3^{a}	13.7^{a}	14.1^{a}	0.766^{a}	8.9^{a}	13.3^{a}
Octanoyl	White sorghum	0.016^{b}	22.0^{c}	1.4^{c}	13.9^{c}	26.7^{c}	33.0^{d}	24.4^{d}	1.569^{b}	15.1^{c}	26.7^{c}
	Brown sorghum	0.016^{b}	22.3^{c}	0.6^{a}	18.1^{d}	49.0^{e}	77.8^{e}	27.9^{e}	3.494^{c}	17.1^{c}	49.0^{e}
	Cassava	0.050^{c}	18.2^{a}	1.4^{c}	22.5^{e}	34.6^{d}	74.8^{e}	18.6^{c}	5.597^{d}	13.1^{b}	34.6^{d}
Acetic anhydride	White sorghum	0.016^{b}	19.7^{b}	0.6^{a}	1.7^{a}	16.4^{b}	16.8^{b}	16.4^{b}	0.731^{a}	11.1^{b}	16.4^{b}
	Brown sorghum	0.016^{b}	17.3^{a}	0.7^{a}	1.7^{a}	17.5^{b}	18.0^{c}	18.4^{c}	0.725^{a}	11.9^{b}	17.5^{b}
	Cassava	0.016^{b}	16.4^{a}	4.7^{e}	2.1^{a}	13.0^{a}	13.5^{a}	14.0^{a}	0.814^{a}	8.5^{a}	13.0^{a}
Acetic acid	White sorghum	0.000^{a}	21.6^{c}	0.6^{a}	1.7^{a}	16.0^{b}	16.4^{b}	16.3^{b}	0.700^{a}	11.0^{b}	16.0^{b}
	Brown sorghum	0.000^{a}	20.4^{b}	2.9^{d}	1.3^{a}	16.0^{b}	16.4^{b}	16.3^{b}	0.699^{a}	11.2^{b}	16.0^{b}
	Cassava	0.000^{a}	19.5^{b}	2.4^{d}	3.2^{b}	13.7^{a}	14.4^{a}	14.2^{a}	0.894^{a}	8.7^{a}	13.7^{a}

DS: degree of substitution; AM: amylose; RS: resistant starch; SD: starch damage.
Means with different letters within the same column indicate significant differences among samples (p<0.05).

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Table 2. Crystallinity degree, oil and water absorption and thermal properties of native and chemically modified sorghum and cassava starch.

Modifications	Starch source	CD (%)	OAC	WAC	ΔH (J/g)	To (°C)	Peak Width (°C)
Native	White sorghum	24.4^{c}	2.03^{a}	2.04^{a}	6.86^{b}	67.87^{b}	7.14^{d}
	Brown sorghum	24.6^{c}	2.12^{b}	2.03^{a}	8.75^{c}	68.19^{b}	7.84^{e}
	Cassava	24.5^{c}	2.32^{c}	2.03^{a}	8.78^{c}	64.29^{a}	10.63^{h}
Octanoil	White sorghum	16.2^{a}	2.40^{c}	2.74^{e}	4.57^{a}	68.56^{b}	6.78^{c}
	Brown sorghum	14.7^{a}	2.67^{d}	2.87^{e}	5.90^{a}	67.26^{b}	7.16^{d}
	Cassava	9.6^{a}	2.35^{c}	3.04^{f}	5.46^{a}	65.22^{a}	9.97^{g}
Acetic anhydride	White sorghum	21.2^{b}	2.21^{b}	1.98^{a}	10.56^{d}	66.62^{b}	7.19^{d}
	Brown sorghum	21.8^{b}	2.05^{a}	2.12^{b}	9.35^{c}	65.13^{a}	7.22^{d}
	Cassava	21.6^{b}	2.34^{c}	2.11^{b}	8.09^{c}	67.62^{b}	9.18^{f}
Acetic acid	White sorghum	21.0^{b}	2.25^{b}	2.31^{d}	7.42^{b}	69.44^{b}	5.70^{a}
	Brown sorghum	21.8^{b}	2.17^{b}	2.22^{c}	8.33^{c}	72.29^{c}	6.44^{b}
	Cassava	21.4^{b}	2.37^{c}	2.09^{b}	12.22^{e}	65.14^{a}	11.10^{i}

OAC= Oil absorption, WAC= water absorption (both expressed as g of absorbed solvent by g of sample), To = onset temperature, ΔH = enthalpy change. Means with different letters within the same column indicate significant differences among samples (p<0.05).

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